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Model comparison to evaluate a shell quality bio-complex in layer hens

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Abstract

Reducing the incidence of egg shell breakage is an important selection goal in egg layer hens breeding. Breaking strength provides an indicator of static shell resistance correlated with shell thickness. Acoustic egg tests combine shell's resonance profile with egg mass to calculate dynamic stiffness (KDyn) a quantitative indicator of integral shell resistance, and a novel direct detection of both cracks and micro-cracks (MCr) making it possible for use in selection programs aiming improvement of shell quality. A shell quality bio-complex was defined to improve overall shell quality, including: breaking strength at equator (BSe) and poles (BSp), KDyn, and MCr, on multiple eggs/hen-age. A total of 81,667; 101,113; and 72,462 records from 4 generations of three pure lines were evaluated. Two models were tested in the brown-egg line: I) four-trait linear repeatability model and II) three-trait linear (BS, KDyn)-threshold (MCr) in the three lines. Models were implemented with AIREMLF90 and THRGIBBS1F90. Heritability and repeatability (Model I) estimates were: h2 = 0.14, 0.18, 0.33, and 0.02; r = 0.16, 0.28, 0.43, and 0.03 for BSe, BSp, KDyn, and MCr, respectively. Corresponding values in White Plymouth Rock were h2 = 0.14, 0.17, 0.33, and 0.02; r = 0.21, 0.33, 0.44, and 0.04, and in White Leghorn were h2 = 0.14, 0.23, 0.36, and 0.02; r = 0.24, 0.38, 0.52, and 0.02. Genetic correlations between BSe and BSp were between 0.51 and 0.68. The BS traits were moderately correlated with KDyn (+0.23 to +0.51), and tended to be negatively correlated with MCr. KDyn, and MCr (−0.46 to −0.62). Model II had similar results; except for increased h2 = 0.06 and r = 0.09 for MCr. Results indicate that BSe and BSp are different traits; while incidence of MCr is low in heritable but showed negative genetic correlations with the other traits. This makes MCr unsuitable for direct selection; but favors indirect selection against MCr via BSe, BSp, and KDyn for a holistic selection to improve shell quality, in particular to achieve the ultimate goal, reduction of egg breakage.

Keywords

layer hen, shell quality, egg quality, selection, genetic parameters

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Comments

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Model comparison to evaluate a shell quality bio-complex in layer hens

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ABSTRACT Reducing the incidence of egg shell breakage is an important selection goal in egg layer hens breeding. Breaking strength provides an indicator of static shell resistance correlated with shell thickness. Acoustic egg tests combine shell's resonance profile with egg mass to calculate dynamic stiffness (KDyn) a quantitative indicator of integral shell resistance, and a novel direct detection of both cracks and micro-cracks (MCr) making it possible for use in selection programs aiming improvement of shell quality. A shell quality bio-complex was defined to improve overall shell quality, including: breaking strength at equator (BSe) and poles (BSp), KDyn, and MCr, on multiple eggs/hen-age. A total of 81,667; 101,113; and 72,462 records from 4 generations of three pure lines were evaluated. Two models were tested in the brown-egg line: I) four-trait linear repeatability model and II) three-trait linear (BS, KDyn)-threshold (MCr) in the three lines. Models were implemented with AIREMLF90 and THRGIBBS1F90. Heritability and repeatability (Model I) estimates were: $h^2 = 0.14$, 0.18, 0.33, and 0.02; r = 0.16, 0.28, 0.43, and 0.03 for BSe, BSp, KDyn, and MCr, respectively. Corresponding values in White Plymouth Rock were $h^2 = 0.14$, 0.17, 0.33, and 0.02; r = 0.21, 0.33, 0.44, and 0.04, and in White Leghorn were $h^2 = 0.14$, 0.23, 0.36, and 0.02; r = 0.24, 0.38, 0.52, and 0.02. Genetic correlations between BSe and BSp were between 0.51 and 0.68. The BS traits were moderately correlated with KDyn (+0.23 to +0.51), and tended to be negatively correlated with MCr. KDyn, and MCr (−0.46 to −0.62). Model II had similar results; except for increased $h^2 = 0.06$ and r = 0.09 for MCr. Results indicate that BSe and BSp are different traits; while incidence of MCr is low in heritable but showed negative genetic correlations with the other traits. This makes MCr unsuitable for direct selection; but favors indirect selection against MCr via BSe, BSp, and KDyn for a holistic selection to improve shell quality, in particular to achieve the ultimate goal, reduction of egg breakage.

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INTRODUCTION

Eggshell quality is of paramount importance in layer chickens. Improving eggshell resistance and reducing incidence of broken eggs are beneficial not only for profitability but also for food safety, as broken eggs get easily contaminated with microorganisms and can contaminate other eggs and transportation, packing, and distribution equipment. Egg laying cycles are getting longer each year, which places added emphasis on persistency of eggshell quality. Shell quality is important not only for commercial producers (table and industry eggs) but also for breeders due to its relationship with hatchability. Solomon (2010) reviewed traditional selection methods to improve shell quality. In general, these methods can be grouped into destructive vs. non-destructive, and static vs. dynamic. The static measurements using non-destructive methods; such as the deformation test, use the displacement of the shell upon applying pressure that simulates point of impact, without cracking the shell. Destructive ones, such as breaking strength (**BS**), apply blunt force on the surface of the eggshell until it is cracked (Bain, 2005). This latter measurement is highly correlated to shell thickness (Washburn, 1992; Zhang et al., 2005; Blanco et al., 2014). Recently, dynamic methods have been introduced to provide a more comprehensive evaluation of the entire shell area. Among these, the acoustic egg test (**AET**) (Coucke et al., 1999; De Ketelaere et al., 2000, 2004; Bain, 2005; Kemps et al., 2006; Mertens et al., 2006) has been the most widely used. The current version of the AET device uses infrared detection of the egg, which is then rolled while a pearl-ended electromagnetic hammer taps the surface of the egg four times (at 90 degree rotation intervals). The sound produced is received by a microphone, which records the sound waves. The corresponding four-quadrant vibration profiles are combined with the egg mass to calculate the dynamic stiffness (**KDyn**), a quantitative indicator of integral shell resistance based on the a method

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developed by Coucke et al. (1999). This measurement is non-destructive, and has proven to be a good predictor of the occurrence of cracks (Bain et al., 2006a; Amer Eissa, 2009; Mertens, 2009). Poor correlations among vibration profiles indicate poor shell structure or presence of micro-cracks (**MCr**); the frequency spectra is clearly distinct between intact eggs (one dominant peak) and cracked ones (broader frequency range and a heterogeneous pattern) (Amer Eissa, 2009). The ability to detect these micro-cracks is important as egg breakage is one of the most important causes of downgrading eggs during classification, and therefore has economic implications for the industry. Direct or indirect selection against egg breakage must be an integral part of any breeding program to improve egg quality. This is a particularly important challenge as it has been difficult to prove direct relationships between classic shell quality measurements and the incidence of cracked eggs in the field (Thompson et al., 1985; Rodriguez-Navarro et al., 2002).

Dynamic stiffness has been proposed for evaluation and use in selection to improve shell quality especially due to its moderate heritability either by itself (Dunn et al., 2005; Bain et al., 2006b) or in combination with other shell and egg quality traits (Icken et al., 2006; Blanco et al., 2014). However, no published research was found which explored the use of crack detection from the AET device as a measurement for selection. Crack detection is appealing as the ultimate trait to improve shell breakage during egg processing, grading, and distribution. The combined use of BS and AET allowed the definition of a shell quality bio-complex (**SQC**) for a holistic improvement of shell quality. In this SQC, breaking strength taken at egg's equator (BSe) and poles (**BSp**), and AET measurement (KDyn and MCr), were taken at four different ages (with sub-sampling at each time) to represent different stages of the production cycle. Shell quality is known to deteriorate as hens age, in part as a response to increase in egg size (Potts and Washburn, 1985; Washburn, 1992), including an increase in the proportion of cracked eggs (Engstrom et al., 1986); therefore, repeated measurements at different ages must be included for evaluation of overall shell improvement. Measurements can then be analyzed with repeatability models, which treat all records as measurement of the same trait; thus assuming perfect correlation across ages. An improved version of the traditional repeatability model was introduced to account for within (sub-sampling) and across age repeatability of records (Arango et al., 2016; Wolc et al., 2016). Alternatively, records taken at different ages can be treated as distinct traits, and analyzed implementing multiple trait models. The objectives of the study herein were to compare different multiple trait repeatability models to evaluate traits in the SQC for selection of layer chickens. Particular emphasis was placed on the use of the AET for detection of MCr, and the feasibility of the addition of this trait to the bio-complex for improvement of shell quality through selection.

MATERIALS AND METHODS

Data and Traits

A SQC was defined to combine different shell quality measurements, and to optimize their contribution to the overall selection index. For the study herein, data from pure lines of three breeds, representing available biotypes for commercial egg production were studied. Two lines represented brown-egg production (Rhode Island Red, **RIR** and White Plymouth Rock, **WPR**), and one line representing white-eggs (White Leghorn, **WL**). Four-generations of data for each line were used, with a total of 81,667 (RIR); 101,134 (WPR), and 72,462 (WL) SQC records. Data comprised of repeated records (2 to 3 eggs) from each of four ages (approximately 26, 42, 65, and 86 weeks). Hens were individually caged and managed in environmentally controlled houses at research farms used for selection of pure line stocks. Eggs were collected at the farm and transported to Hy-Line International Egg Quality Lab to be processed for internal and external egg quality traits within 24 h of being laid. Each egg was individually identified by hen and evaluation period, and considered as a separated record. Egg weight (**EW**) was determined using a 0.1 g precision scale and the AET evaluation was performed using the Columbus device (Octinion, Leuven, Belgium), which returned the KDyn and MCr detection (yes or no value). The shell BS was measured using either one of two devices: QC-SPA, TSS (York, UK) or Futura FEST device (Lohne, Germany). Two modalities were used, measurement taken at the egg's equator for eggs sampled at ∼26 and 65 wk, and the same measurement taken at the egg's pole for eggs sampled at ∼42 and 86 wk. All other traits were captured at all ages. A cracked egg was coded 100 for the linear analysis and 1 for threshold analyses; while an intact egg was coded as 0 and 2 for the two types of analyses. This made possible to interpret results in terms of percent cracks in the linear scale. When an egg was detected as carrying a MCr, KDyn values are not valid, and were excluded from the data for analysis.

Data Analysis

Model (I) Multiple trait linear model: A four-trait (BSe, BSp, KDyn, and MCr) linear repeatability model, where all traits, including MCr detection, were treated as continuous traits. Fixed effects were contemporary group (hatch within generation), lab station, and the covariates of hen's age and egg weight of the same egg subject to shell quality evaluation. Random effects were animal additive genetic and animal permanent environment, and residual. This model was implemented using AIREMLF90 (Misztal et al., 2015).

Model (II) Multiple trait linear-threshold model: defined crack detection as a categorical trait (yes or no value), and with the linear model assuming continuous variables, a more appropriate distribution could

be considered by implementing a threshold model. For this approach, a three-trait linear-threshold repeatability model: BS, KDyn (linear), and MCr (threshold) were tested in one of the lines (RIR) for comparison with Model I. Exploratory analyses were attempted for implementation of a four-trait linear (BSe-BSp-KDyn)- threshold (MCr) model, but these resulted in convergence and numerical problems (results not shown), due to the pattern of missing data. This was the case because BSp was a new measurement, and data was limited. Therefore, equator and polar records were combined, and a fixed effect was added for BS to account for the direction of the measurement. This allowed for implementation of a three-trait linear-threshold approach, in which, BS and KDyn were treated as continuous traits; while MCr detection was treated considering its categorical distribution (1, 2). Fixed effects were the same as in model I, plus the direction (equator or pole) of the BS measurement. This model was implemented using an Markov Chain Monte Carlo (**MCMC**) Gibbs sampling approach using the THHRGIBBS1F90 program (Misztal et al., 2015), which estimates (co)variance components and genetic parameters in threshold animal mixed models, and allows for any combination of categorical and continuous traits (Lee et al., 2002). Post-Gibbs analyzes were done using POSTGIBBSF90, a program developed by S. Tsuruta (Misztal et al., 2002). The analysis was run as a single chain of 100,000 cycles with a conservative burn-in period of the first 5,000 iterations. Chain stationary stage was monitored by graphical inspection, tracing plots of the sampled values vs. iterations (Kass et al., 1998). Following the burn-in period, summaries of (co)variance components estimates were kept every 20th sample (n = 5,000). Comparison of direct and correlated response, their ratio and the relative efficiency of correlated response (**REC**) to selection were calculated using the formulas by van Vleck et al. (1987).

RESULTS AND DISCUSSION

Overall summary of basic trait statistics is shown in Table 1. Statistical analyzes were carried out for each line separately. The genetic lines used in the study herein represent samples of distinct breeds available for commercial egg production. The objectives of the study were to analyze the multiple traits under study in different populations, not line to line comparisons. In general, means for shell quality traits were similar for all lines, with the exception of a lower BS for the WL line. Incidence of MCr, as detected by the AET device, was similar across lines, and ranged between 7.5 and 9.6%. These values tended to be lower than the 10 to 15% reported in commercial hybrid flocks in group cages (Bain et al., 2006a).

Model I

Estimates of (co)variance components for model I are summarized in Table 2, and corresponding genetic parameters are shown in Table 3. Estimates of heritability for BSe and BSp were relatively low in all lines (0.14 to 0.23), and tended to be slightly greater for the pole vs. equatorial measurement in all lines (0.18 to 0.23) vs. (0.14). Repeatability estimates for BS were low to moderate (0.17 to 0.38) across lines and orientation of measurement. The heritability estimates are similar to those reported by Dunn et al. (2005) in a RIR line using the animal model, for measurements in the equator (0.18), and in the poles at 60 weeks (0.17); greater values were obtained with different methods (sire and dam variance components) and ages. In the same RIR line used by Dunn et al. (2005), Icken et al. (2006) reported a heritability estimate of 0.10 for BS, and indicated that the BSp is preferred due to its higher repeatability, which is in agreement with the results herein. Our results showed lower BS heritability than the ones reported for four lines representing two breeding programs, for which heritabilities were of 0.23 and 0.33 (white-egg lines), and 0.34 and 0.35 (brown-egg lines) as reported by Blanco et al. (2014); however, it was expected as they analyzed data based on hen average records; while we implemented a full repeatability animal model. Our estimates were closer to their estimates when a comparable model was used (J. Arango, unpublished data). In an White Leghorn line selected for shell membrane attachment to the mineral shell, estimates of heritability for all nine shell traits were high (BS = 0.48) using combined data from the base and selected populations, and using Restricted Maximum Likelihood (REML) estimates with canonical transformation (Johansson et al., 1996). More recent estimates of heritability of BS at different ages (32 to 72 wk) using a random regression model ranged from 0.26 to 0.43; but they were measured in a crossbred line between a WL and a blue-egg line (Guo et al., 2015).

Table 1. Summary statistics for shell quality and covariate traits in three lines of layers.

Trait[1]/Line	BSe	BSp	KDyn	MCr	EWt
Rhode Island Red					
N	62,975	9,897	81,667	81,667	81,667
Mean	4,059.9	4,059.8	147.8	8.7	58.6
STDev	992.93	1,032.90	20.14	28.10	4.85
Min	1,001	1,030	40.0	0.0	38.0
Max	6,998	6,991	225.7	100.0	83.0
White Plymouth Rock					
N	85,591	12,805	101,134	101,134	101,134
Mean	4,076.4	4,009.0	134.9	9.6	61.7
STDev	966.62	1,032.90	20.14	29.48	5.6
Min	1,013	1,030	32.1	0.0	37.0
Max	7,085	6,980	241.5	100.0	89.0
White Leghorn					
N	51,811	11,462	72,462	72,462	72,462
Mean	3,821.7	3,228.4	132.1	7.5	61.1
STDev	868.74	1,063.4	19.36	26.33	5.91
Min	903	1,050	40.1	0.0	38.0
Max	6,993	6,090	221.6	100.0	89.0

[1]BSe: Breaking strength equator (gF); BSp: Breaking strength polar (gF); KDyn: Dynamic stiffness (N/cm); MCr: Micro-crack (0, 100 for linear models, 1, 2 for threshold model); EWt: egg weight (g).

Table 2. Estimates of variances[1] and parameters[2] for breaking strength at equator (BSe) or poles (BSp), dynamic stiffness (KDyn) and micro-crack detection (MCr) for model I.

	Rhode Island Red			White Plymouth Rock			White Leghorn		
	σ^2_a	σ^2_{pe}	σ^2_e	σ^2_a	σ^2_{pe}	σ^2_e	σ^2_a	σ^2_{pe}	σ^2_e
BSe	919.2	187.1	5,734.1	960.0	506.0	5,651.0	733.6	539.8	4,086.7
BSp	1,290.1	702.3	5,238.3	1,541.0	1,522.0	6,192.0	1,677.5	1,124.7	4,530.1
KDyn	135.7	39.0	232.1	175.9	60.4	293.9	118.6	51.54	159.4
MCr	15.56	6.37	785.6	14.84	14.3	813.4	15.31	1.46	673.3
	h^2	r		h^2	r		h^2	r	
BSe	0.14	0.16		0.14	0.21		0.14	0.24	
BSp	0.18	0.28		0.17	0.33		0.23	0.38	
KDyn	0.33	0.43		0.33	0.44		0.36	0.52	
MCr	0.02	0.03		0.02	0.04		0.02	0.02	

[1] σ^2a = direct additive genetic variance; σ^2pe = permanent environmental variance; σ^2e = residual variance.
[2] h^2 = heritability; r = repeatability.

Table 3. Estimates of genetic (above diagonal) and permanent environmental correlations (below diagonal) for breaking strength at equator (BSe) or poles (BSp), dynamic stiffness (KDyn), and micro-crack detection (MCr) for model I.

	Rhode Island Red				White Plymouth Rock				White Leghorn			
	BSe	BSp	KDyn	MCr	BSe	BSp	KDyn	MCr	BSe	BSp	KDyn	MCr
BSe	-	0.68	0.28	−0.27	-	0.61	0.23	0.03	-	0.51	0.27	−0.23
BSp	0.80	-	0.31	−0.42	0.40	-	0.31	−0.43	0.23	-	0.51	−0.60
KDyn	0.78	0.69	-	−0.46	0.45	0.37	-	−0.61	0.53	0.21	-	−0.62
MCr	−0.29	0.07	−0.37	-	−0.28	−0.19	−0.29	-	−0.68	−0.51	−0.87	-

Estimates of heritability and repeatability for KDyn were substantially greater than those found for BS. Heritability was 0.33 in brown-egg lines and 0.36 in the White Leghorn line (Table 2). Corresponding repeatability values ranged from 0.43 to 0.52. These values are in line with other studies that also found greater proportion of heritable variation for Kdyn as compared with breaking force (Dunn et al., 2005; Icken et al., 2006; Blanco et al., 2014). In a RIR line, Dunn et al. (2005) reported estimates of heritability ranging from 0.33 to 0.58 (estimates tended to be lower using the sire variance component, and were 0.53 with the animal model). Using the same line, Icken et al. (2006) reported a value of 0.40. Blanco et al. (2014), in the four-line study mentioned above, reported for Kdyn heritability values of 0.35 and 0.43 in WL and of 0.70 and 0.65 in RIR and WPR. All the comparable studies used hen-average records; so, it is not surprising that our estimates of repeatability for KDyn were closer to their estimates of heritability.

Estimates of heritability for MCr percent (Table 2) were consistently low across lines (0.02), and values had low repeatability (0.02 to 0.04). These values suggest that detection of micro-cracks is highly dependable on non-genetic factors; for instance random environmental effects such as the ones generated by eggs receiving impacts during laying or rolling out of the cage. This renders MCr as an unreliable trait for direct selection to reduce egg breakage in commercial populations. Our results represent the first study that analyzed the incidence of cracks as detected by the AET device as a potential trait for selection. A more laborious candling method of crack detection was used to estimate the frequency of cracks in two generation of a WL line after 8 generations of selection for egg number and egg size (Engstrom et al., 1986). Their estimates of heritability for frequency of cracks were low (0.11) at two different ages (34 to 49 and 57 to 63 weeks) when cracks were measured at candling in an egg grading station, and values were transformed to a logarithmic scale. Greater heritability values were obtained when cracks were measured in front of the cages in older hens, and classified as start, line, or other type of cracks. The incidence of cracks in Engstrom et al. (1986) study was large (on average 31.2%), which may be the reason for obtaining a moderate heritability estimate of 0.43. Johansson et al. (1996) obtained a high estimate of heritability for the frequency of cracked eggs (0.58) in the WL selection experiment mentioned earlier; but in this particular study, no mention was made about the way cracks were measured, and heritability was high for all shell traits. Wolc et al. (2005) also used linear and threshold animal models to analyze egg cracks, using hen average at different ages in two Rhode Island lines (Brown and White). Their estimates of heritability using the linear model were low (0 to 0.097), averaging 0.092 and 0.069 in the two lines; corresponding values with the threshold model were larger (0 to 0.794), averaging 0.618 and 0.499. Other studies that tried to use the reciprocal trait (i.e., percent of intact eggs) also reported low heritability estimates (Grunder et al., 1989). At that time, selecting for percent of intact eggs was discouraged not only due to low heritability but also because of the impractical nature of the measurement; thus favoring an

indirect selection to reduce egg breakage (Grunder et al., 1989, 1991).

The consistently greater heritability estimates for KDyn stiffness found here are in agreement with other research and favor its use for selection (Dunn et al., 2005; Icken et al., 2006; Blanco et al., 2014). The reasons for a more heritable proportion of heritable variation for KDyn than for other shell quality traits is not well known; but it may be partly due to some traits being used for a long time in selection programs (Washburn, 1992 for a literature review), therefore genetic variation has eroded; whereas AET is a novel technology for which genetic variation has been utilized for only a short time in breeding populations.

Genetic correlations (Table 3) between BSe and BSp were similar across lines, ranging from +0.51 to +0.68. The difference in ages at poles and equatorial measurements could have contributed to the deviation of the correlation from one in addition to direction of the measurement itself. Permanent environmental correlations were more variable and ranged from +0.23 to +0.80. No estimates of genetic correlations between the two directions of measurement were found in the literature, however, Cordts et al. (2002) reported relatively low phenotypic correlations between BS in the equator and poles (+0.21 to +0.31) in two lines. Genetic correlations between both BS measurements and KDyn were relatively consistent across lines and were moderate (+0.23 to +0.51) indicating that these two traits capture distinct biological aspects of shell quality. Corresponding permanent environmental correlations were more widely distributed ranging from +0.21 to +0.78, indicating that there are similarities between measurements of the same hen; but that not all are genetically determined. Dunn et al. (2005) found a genetic correlation of Kdyn with equatorial BS of +0.49 (sire estimate) and +0.40 (dam estimate) in a RIR line. In the same study, estimates of genetic correlations at different ages were presented between Kdyn and BS in the poles, ranging from +0.36 to +0.53 (sire estimate) and +0.40 to +0.69 (dam estimate). Icken et al. (2006) reported a genetic correlation between Kdyn and BS of +0.57. Blanco et al. (2014) also reported intermediate correlations between these two traits in four lines (+0.40 to +0.61). So, in general, there is research agreement of positive and moderate to intermediate genetic correlations between KDyn and BS, indicating that selecting by a static-destructive and a dynamic-non-destructive measure of shell quality is not antagonistic, and combing both measurements should lead to a better improvement of overall shell quality.

Genetic correlations between MCr detection and other shell quality traits were negative across lines and traits (Table 3). For BSe in WPR line it was about zero (0.03) whereas all other estimates ranged from −0.23 to −0.62. Results herein are consistent with the ones obtained in different WL and WPR lines, used for initial AET testing (J. Arango, unpublished data). No genetic correlations between egg cracks (as detected by the AET device) and other shell quality traits were found in the literature. However, a clear relationship between KDyn and the probability of cracks was presented by Bain et al. (2006a). Studies that measured a reciprocal trait (percent of intact eggs) found genetic correlations of similar magnitude but reverse sign as excepted (Grunder et al., 1989, 1991). Genetic correlation between early breaking strength (42 to 45 wk) and late intact eggs (67 to 68 wk) were of +0.47 (covariance analysis) and +0.59 (dam-daughter regression) and corresponding values for late traits were higher (+0.60 and +0.92) (Grunder et al., 1989). Johansson et al. (1996) reported a negative genetic correlation between frequency of cracked eggs and five shell quality traits in WL. Their value for the correlation with BS was −0.40, which is in agreement with our findings.

In general, the above results indicate that greater values for BS and KDyn are genetically associated with lesser incidence of micro-cracks as detected by the AET device. Also, in brown-egg lines the genetic correlations between MCr and KDyn were of greater magnitude than the permanent environmental ones (−0.46 vs. −0.37 in RIR, and −0.61 vs. −0.29 in WPR). In the WL line, both were of large magnitude (−0.62 and −0.87). These results demonstrate the strong negative, but favorable, relationship between these two traits, and document an important genetic component. All the results obtained indicate that when developing the SQC, including both BS and KDyn in the selection index will help in decreasing the incidence of cracks. Due to the low genetic variation for incidence of cracks and the much larger heritability of the other two traits, predicted correlated response to selection is greater than direct response for MCr detection. The concept of relative efficiency of correlated response was introduced by van Vleck et al. (1987). It allows comparing direct vs. correlated response to selection. If the REC value is greater than 1.0, then the correlated response is more efficient to improve the target trait than the response to direct selection. Table 4 shows the expected response to direct selection on MCr and KDyn, the correlated response by selection on Kdyn, and the corresponding REC value. It is shown that indirect response in MCr by selecting for KDyn is over two times (2.12 to 2.50) greater than the direct response achievable by direct selection against micro-cracks in the three lines. Similar

Table 4. Direct (D) versus correlated (C) response, their ratio and relative efficiency of correlated response (REC) to selection for dynamic stiffness (KDyn) and micro-crack detection (MCr) in three lines[1].

	D_KDyn	D_MCr	C_MCr/KDyn	REC
RIR	6.928	0.508	−1.101	−2.17
WPR	8.196	0.566	−1.201	−2.12
WL	6.533	0.583	−1.459	−2.50

[1]RIR = Rhode Island Red; WPR = White Plymouth Rock; WL = White Leghorn.

Table 5. Estimates of variances and covariances for breaking strength (BSe), dynamic stiffness (KDyn), and micro-crack detection (MCr) for model II in the Rhode Island Line.

	Additive genetic			Permanent environmental			Residual effects		
Variance	Mean	HPD[1] (95%)	ESS[2]	Mean	HPD[1] (95%)	ESS[2]	Mean	HPD[1] (95%)	ESS[2]
BS	914.5	814.5 to 1019.0	166	298.6	233.4 to 361.4	135	6132.9	6,074 to 6,182	4,080
KDyn	143.36	131.9 to 156.2	554	51.54	45.44 to 58.76	490	185.27	183.0 to 187.7	4,152
MCr	0.064	0049 to 0.079	117	0.035	0.017 to 0.053	25	1.000	0.989 to 1.011	4,500
Covariance									
BS-KDyn	119.3	94.3 to 146.0	386	74.37	59.50 to 89.48	338	211.5	202.0 to 221.1	3,997
BS-MCr	−2.76	−3.77 to −1.83	77	−0.58	−1.35 to 0.19	45	−14.68	−15.74 to −13.68	1,412
KDyn-MCr	−1.84	−2.17 to −1.49	271	−0.41	−0.65 to -0.18	205	Na	Na	Na

[1]HPD = Higest posterior density (95%) interval.
[2]ESS = Effective sample size.
Na: Not available.

Table 6. Estimates of parameters (heritability and *repeatability*)[1] and genetic (above diagonal) and permanent environmental correlations (below diagonal) for breaking strength (BS), dynamic stiffness (KDyn), and micro-crack detection (MCr) for model II in the Rhode Island Line.

	BS	KDyn	MCr
BS	0.13 (*0.17*)	0.34	−0.36
KDyn	0.60	0.38 (*0.51*)	−0.61
MCr	−0.18	−0.31	0.06 (*0.09*)

[1]Diagonal: Estimate of heritability (*repeatability*).

results were obtained in a different WL line during initial testing of the AET technology (results not shown).

Model II

Table 5 presents estimates of (co)variance components for model II, their means, highest posterior density intervals (95%), and effective sample size (**ESS**). The last one is a common diagnostic of behavior of MCMC chains. It represents the match between proposal and target distribution (i.e., if the proposal distribution is suitably chosen), and has become the standard diagnostics due to its simplicity, intuitive interpretation, and its robustness (Nowozin, 2015). Parameter estimates for this model are summarized in Table 6. The posterior distributions of the additive genetic and residual variances are illustrated in Figure 1. The chosen length of the MCMC chains sufficed for estimation of the (co)variance components, ESS were adequate for all parameters, especially additive (ESS = 77 to 554) and residual (ESS = 1412 to 4500) variances and covariances across traits. The posterior distributions for additive and residual variances were acceptably symmetric (Figure 1), and showed a good agreement between the mode and mean of the posterior distributions. This was the case even for the categorical trait whose distributions were comparable to those of the continuous traits. Heritability estimates (based on the mean values in Table 5) were 0.13, 0.38, and 0.06 for BS, KDyn, and MCr, respectively. The corresponding repeatability estimates were 0.17, 0.51, and 0.09, respectively. These estimates were similar to those obtained with model I for BS, slightly larger for KDyn and larger for MCr. Although the estimates of heritability and repeatability for MCr using the threshold model (II) were still low (0.06 and 0.09, respectively), they represent 300% increase when compared with the corresponding values using the linear model I. In Model II, the mean genetic correlation of MCr with BS and KDyn were of −0.36 and −0.61. These values were slightly greater than the average ones for BSe and BSp (−0.345), and greater than the one estimated with KDyn (−0.46), using model I for the RIR. The permanent environmental correlations between MCr and the other traits were also negative (−0.18 with BS and −0.31 with Kdyn); but of lesser magnitude than the genetic ones. These results indicate that greater values of KDyn and BS are genetically associated with lesser incidence of cracks, as expected and that the nature of such relationship is mainly genetic. No other reports of estimates of heritability or repeatability for crack detection using threshold or other non-linear models were found in the literature.

Studies of genetic determination of egg breakage are scarce in the literature. Engstrom et al. (1986) estimated the incidence of cracked eggs, and Grunder et al. (1989, 1991) defined a reciprocal trait, percent intact eggs. The last study discarded the idea of direct selection to reduce egg breakage due to low heritability and laborious measure required; then favoring indirect selection. In the case herein, the last argument is not valid anymore as the AET device provides quick crack detection in a practical way as a co-product of the acoustic profile analysis during calculation of KDyn; so, no additional measurement is required. However, we also propose the improvement of eggshell integrity by means of indirect selection due to a combination of low heritability of MCr (even with the threshold model), and a favorable genetic correlation with the other two traits in the shell bio-complex (BS and KDyn), in particular with dynamic stiffness. When the calculations carried out in Table 4 for Model I in the RIR line are implemented using values obtained with Model II a REC value of 1.523 is estimated. This indicates that the correlated response in MCr by selecting for KDyn is 1.52 times greater

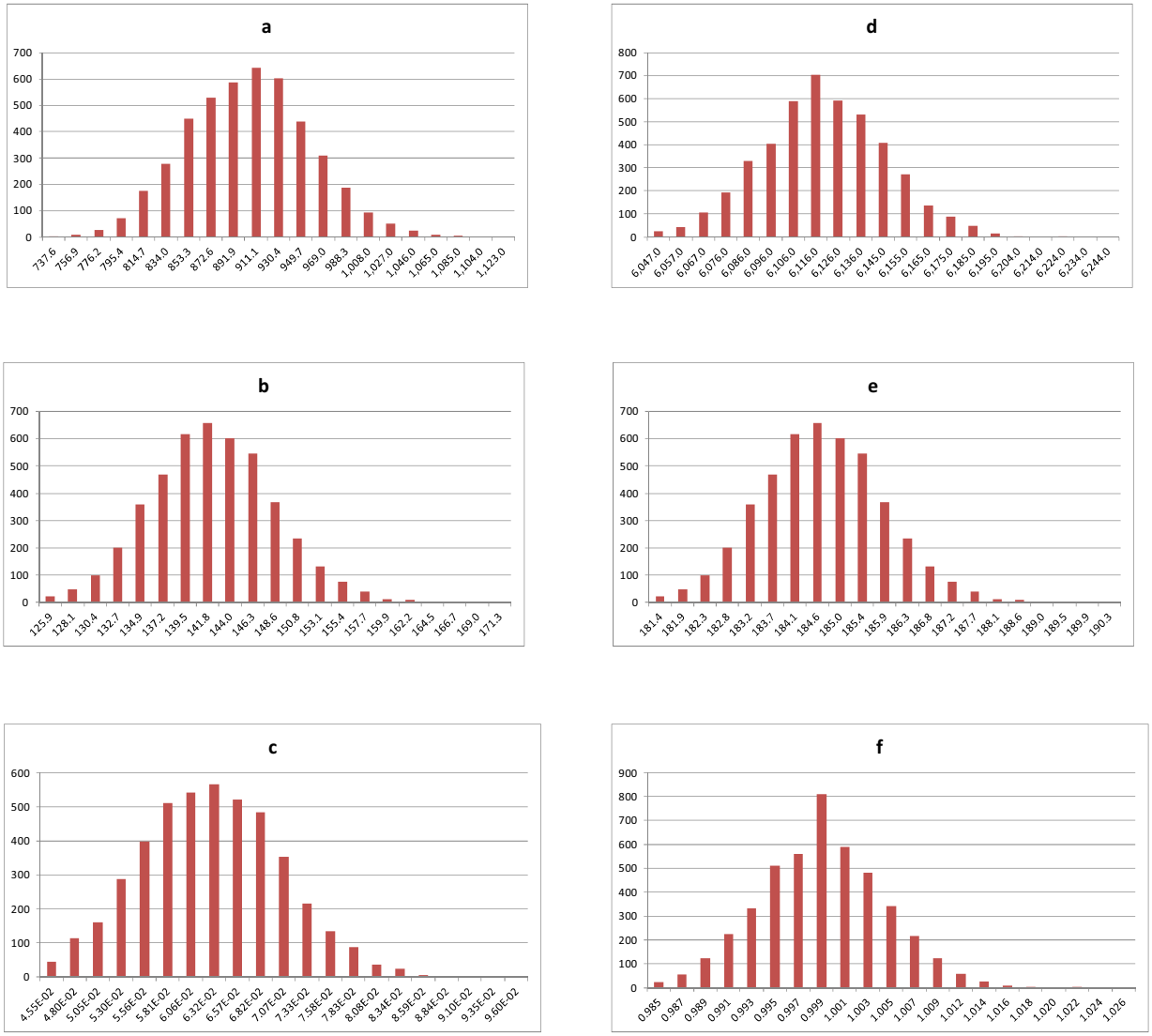

Figure 1. Posterior distributions of additive genetic (left) and residual (right) variances for breaking strength (a, d), dynamic stiffness (b, e), and crack detection (c, f).

than the direct response achievable by only direct selection on MCr in this line. This is of a lesser magnitude than the REC obtained with the linear Model I of 2.17 (Table 4). In other words, the increment in heritability for MCr obtained with the threshold model was not high enough to offset the benefit of the indirect selection using the correlated response with KDyn (i.e., REC < 1.0) to reduce the incidence of eggshell cracks.

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